

Application. No. 09/876,348  
Amendment dated August 5, 2005  
Reply to Office Action of May 11, 2005

**REMARKS/ARGUMENTS**

Reconsideration of the above-identified application is respectfully requested in view of the amendments to the claims and the following remarks.

Claims 4, 7, 10, 33 and 34 have been cancelled.

Claims 1 and 6 have been amended.

Claims 1-3, 5-6, 8-9, and 11-32 remain in the case.

No revision of inventorship is necessary in the present application.

**Support for the Amendment**

Claims 1 and 6 have been amended to include the language suggested by Examiner Robinson, in the May 11, 2005 Office Action. No other changes to the claims have been made.

The specification has been amended to correct the informalities noted by Examiner Robinson, in the May 11, 2005 Office Action. These changes include changes to the Brief Description to correct the format of the sequence notation, changes to the Brief Description to reflect the currently revised drawings, and to correct page 102, line 34. Changes to other portions of the specification have also been made to correct the format of the sequence notation. Additional changes have been made to correct spelling informalities. No new material has been added.

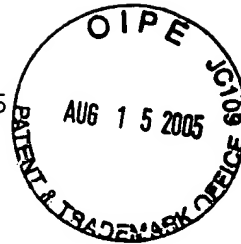
The figures 4.16-4.18, 8.43, and 8.44 have been revised to eliminated color and color references, using asterisks, letters, and outline boxes in place of color. No new material has been added.

### Invention Summary

Thermal hysteresis proteins (THPs) also known as antifreeze proteins are known to lower the non-equilibrium freezing point of water without lowering the melting point (equilibrium freezing point). The present invention details relative recrystallization inhibition behavior of thermal hysteresis proteins. In particular, extremely dilute solutions of THPs have been shown to inhibit the recrystallization of fine-grained ice samples in a concentration-dependent manner. The high sensitivity of RI to the presence of THPs led Applicants to the present invention, as recited in the amended claims, which is a quantitative assay of THP activity using the recrystallization inhibition behavior. The extent of recrystallization in a fine-grained ice sample is quantified by estimating mean largest cross-sectional area for ice grains in the sample, thus providing the basis for a numerical assessment of RI. A number of different assay characteristics is addressed and described in the specification, including specificity of the RI assay with respect to THP, ice grain size homogeneity within RI ice samples, RI assay sensitivities, applications of the assay, and assay automation.

As defined in currently amended claim 1, the invention particularly defines a relative recrystallization inhibition analysis method for determining the presence, relative concentration, and activity of THPs. A test solution made of a proteinaceous composition in a solvent is flash frozen; the temperature of the frozen solution is raised to an appropriate annealing temperature that allows for a partial melt, while limiting heterogeneity in ice grain sizes within the solution. The frozen solution is maintained at the annealing temperature for a length of time sufficient to allow for ice recrystallization. Changes in ice crystal grain size are monitored over time; and the presence of functional THPs (while reducing the effect of non-specific proteins) in the solution is determined by measuring ice crystal grain sizes relative to a control solution.

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Conclusion

In view of the above remarks and amendment to the claims, to the figures, and to the specification, Applicants believe they have overcome the objections noted. Applicants request reconsideration of this application and allowance of pending claims 1-3, 5-6, 8-9, and 11-32.

Respectfully submitted,  
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A handwritten signature of Mark Levy, consisting of a stylized "M" and "L" followed by a horizontal line.

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(Date of Deposit)

8/9/05  
Mark Levy, Reg. No. 29,188 (Date)

Attachment: Appendix with amended and annotated drawing Figures.

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**Amendments to the Drawings:**

The attached sheet of drawings includes changes to Fig. 2.6c. This sheet replaces the original sheet including Fig. 2.6c. This figure now shows proper spelling of "residue" in the heading beginning with "A". The color highlighting has removed from Figures 4.16, 4.17, 4.18, 8.43, and 8.44, and replaced with arrows, asterisks, and boxes. See annotated Figures 4.16, 4.17, 4.18, 8.43, and 8.44.

Attachment: Replacement Sheets for figures: 2.6c, 4.16, 4.17, 4.18, 8.43, and 8.44. Annotated sheets for 2.6c, 4.16, 4.17, 4.18, 8.43, and 8.44.

A. Mature Tm 13.17 amino acid residue

1 LTEAQIEKLN KISKKCQNES GVSQEIIITKA RNGDWEDDPK LKRQVFCVAR  
1 NAGLATESGE VVVDVLREKV RKVTDNDEET EKIINKCAVK RDTVEETVFN  
1 TFKCVMKNKP KFSPVD

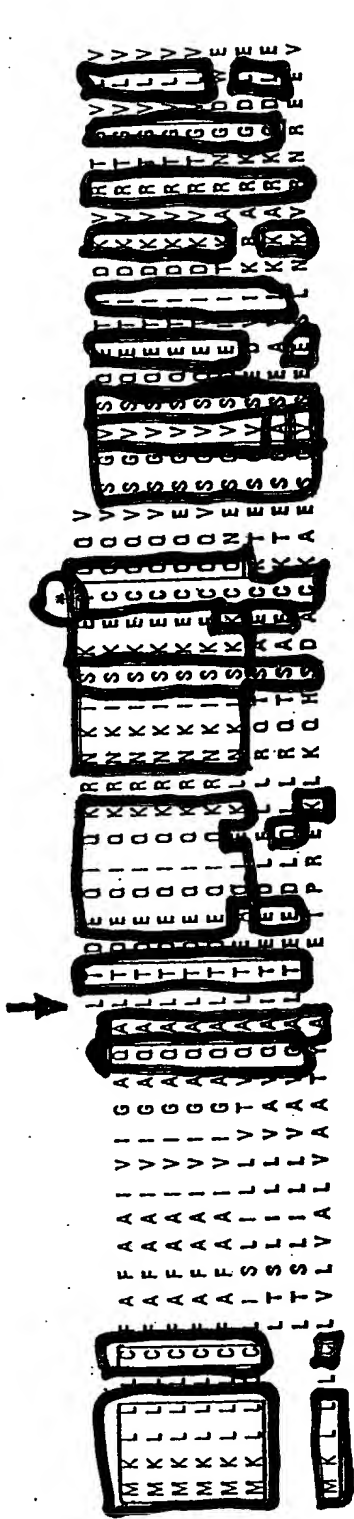
B. Summary of the composition analysis for the mature Tm 13.17 sequence:

<u>Residue</u>	<u>Number</u>	<u>Mole Percent</u>
A = Ala	6	5.172
B = Asx	0	0.000
C = Cys	4	3.448
D = Asp	8	6.897
E = Glu	13	11.207
F = Phe	4	3.448
G = Gly	4	3.448
H = His	0	0.000
I = Ile	6	5.172
K =Lys	16	13.793
L = Leu	5	4.310
M = Met	1	0.862
N = Asn	8	6.897
P = Pro	3	2.586
Q = Gln	4	3.448
R = Arg	6	5.172
S = Ser	5	4.310
T = Thr	8	6.897
V =Val	14	12.069
W = Trp	1	0.862
Y = Tyr	0	0.000
Z = Glx	0	0.000

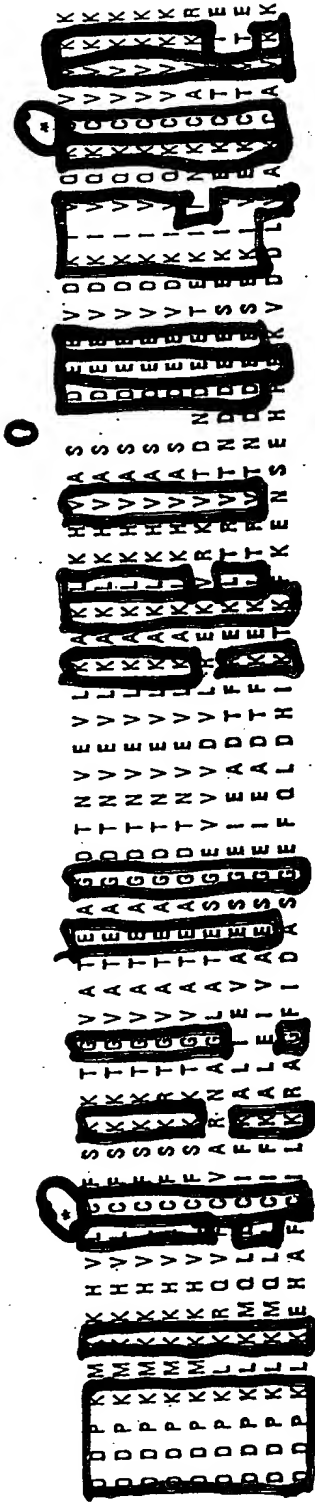
Molecular weight = 13171.96; Residues = 116; Average Residue Weight = 113.551

Charge = 1; Isoelectric point = 7.74.

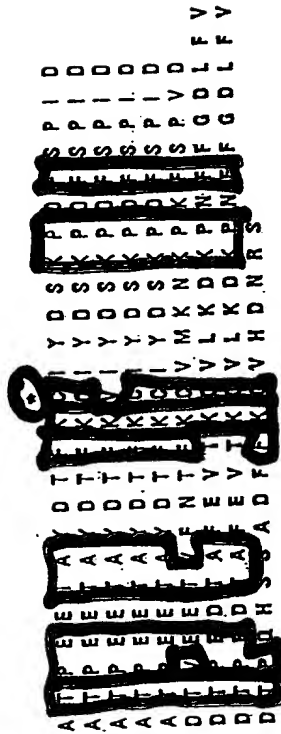
FIG 2.6c



Tm 12.88  
2-2  
2-3  
3-4  
3-8  
7-5  
Tm 13.17  
B1  
B2  
AFP-3



2-2  
2-3  
3-4  
3-8  
7-5  
Tm 13.17  
B1  
B2  
AFP-3



2-2  
2-3  
3-4  
3-8  
7-5  
Tm 13.17  
B1  
B2  
AFP-3

*Fig. 4.16*

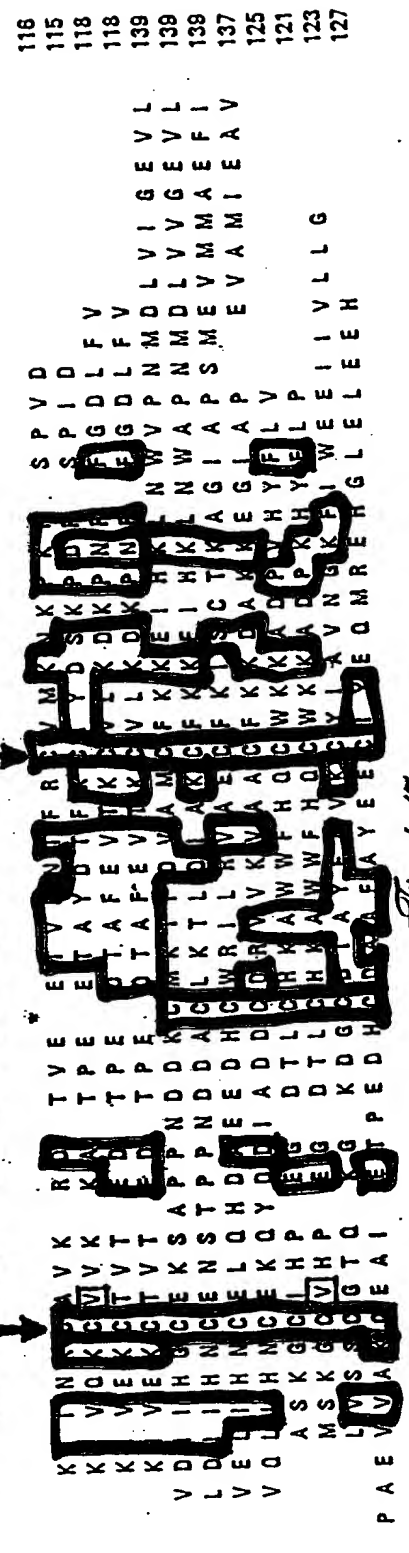
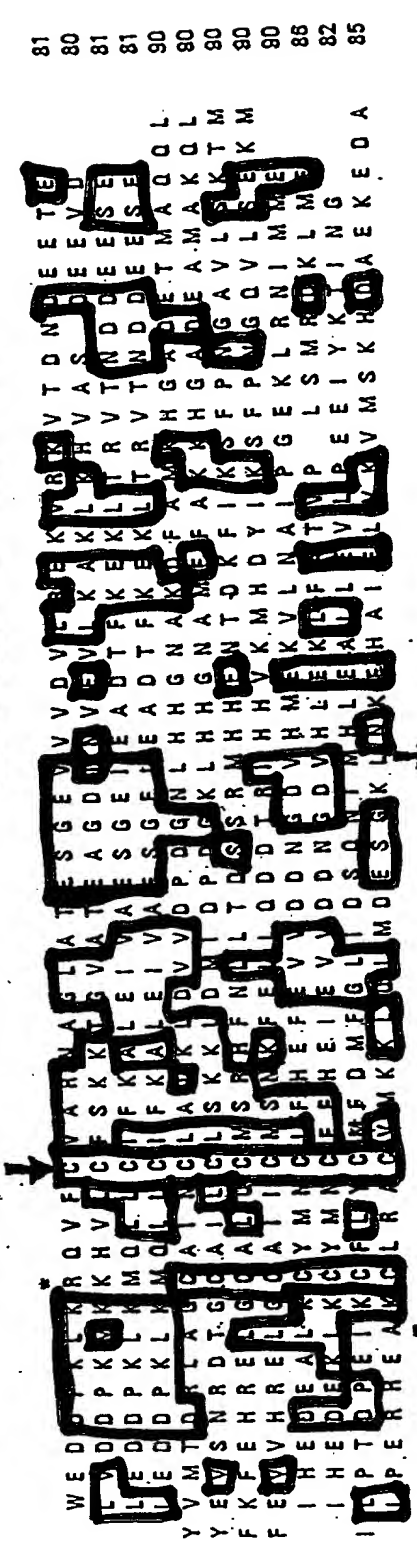
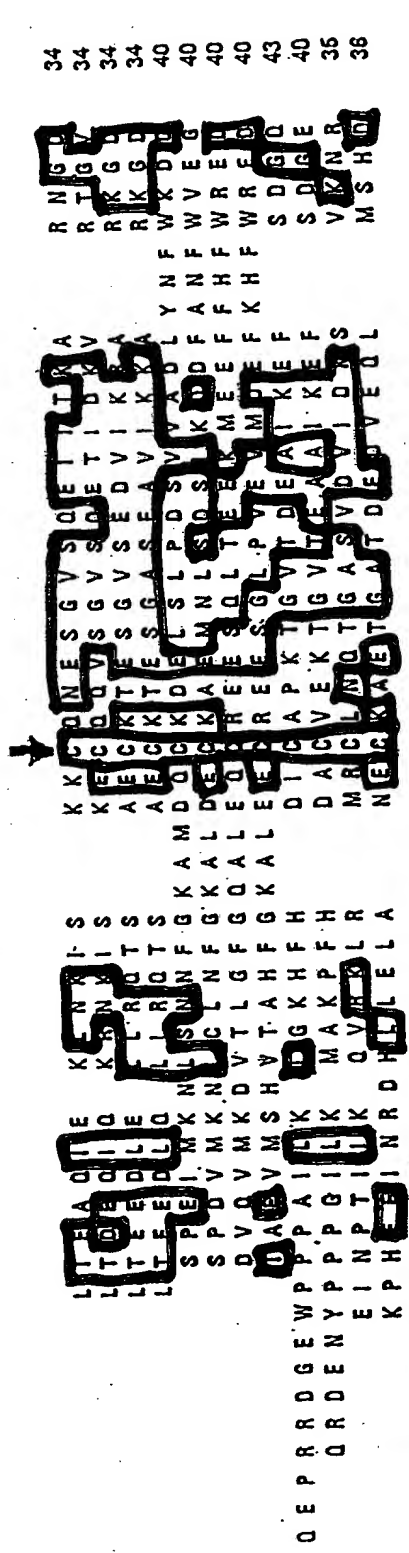


Fig. 4.17

**Tm 12.88**

## 2.2

2-3

卩

3-8

7-5

三

25

78

**ATF**

[illegible]

2-2

## 2-3

43

三

## 7-5

**Time**

18

82

**AF**

[illegible]

2.2

## 2.3

33

3.

7-2

in

18

22

**AT**

[illegible]

*Fig. 4.18*



### DNA sequence of Tm 13.17 cDNA clone

B E  
a c  
m o  
H R  
I I

1 AGTGGATCCAAAGAATTTCGGCACGAGACTACTAAGATGAAGTTGCTCTGTTGTCTAATCT  
M K L L C C L I S

61 CCCTCATTCTGTTGGTCACAGTTCAGGCCCTGACCGAGGCCACAAATGAGAACTGAACA  
L I L L V T V Q A L T E A Q I E K L N K

121 AGATCAGCAAAAAATGTCAAATGAAAGTGAAGTCCGCAAGC GATCATAACCAAAGCTC  
I S K K C Q N E S G V S Q E I I T K A R

181 GCAACGGTGACTGGGAGGACGATCCTAAACTGAAACGCCAAGTTTTTTGCGTGGCCAGGA  
N G D W E D D P K L K R Q V F C V A R N

241 ACGCCGGTCTGGCCACGGAATCGGGAGAGGTGGTGGTTCGACGTGTTGAGGGAGAAGGTGA  
A G L A T E S G E V V V D V L R E K V R

301 GGAAGGTCACTGACAACGACGAAGAAACTGAAGAAATCATCAATAGTCCCGGACAAAG  
K V T D N D E E T E K I I N K C A V K R

361 GAGAACTGTTGAGGAGACGGTGTTCATACTTTCAAATGTGTCATGAAAAACAAGCCAA  
D T V E E T V F N T F K C V M K N K P K

421 AGTATCACTGTTGATGTAACCACCAGCACTAGTAGATGGTTCAAATGGTGTGCTTTAC  
F S P V D \*

X  
h  
o  
I

481 ATATAAAAAATAAAGTGTTTCTGATGTAAAAAATAAAAAAAAAAAAAAAAAAAAACTCG  
polyadenylation signal poly (A) tail (26)

537 AGAGTATTCTAGAGCGGCCCGGGCCCATCGTTTTCCACCC

1 GGCACGAGCAAAAATGAAACTCCTCTTGTGCTTTGCGTTCGCGCCG  
                                   M K L L L C F A F A A  
 47 ATCGATCGGAGCTCAGGCTCTCACCAGGATATACAGAAA  
    I V I G A Q A L I D E Q I Q K  
 92 AGGAACAAGATCAGCAAGAATGCCAGCAGGTGTCCGGAGTGTCC  
    R N K I S K E C Q Q V S G V S  
 137 CAAGAGACGATCGACAAAGTCCGCACAGGTGTCTTGGTCGATGAT  
    Q E T I D K V R T G V L V D D  
 182 CCCAAAATGAAGAAGCACGTCCTCTGCTTCTCGAAGAAAACCTGGA  
    P K M K K H V L C F S K K T G  
 226 GTGGCAACCGAAGCCGGAGACACCAATGTGGAGGTACTCAAAGCC  
    V A T E A G D T N V E V L K A  
 271 AAGCTGAAGCATGTGGCCAGCGACGAAGAGGTGGACAAGATCGTG  
    K L K H V A S D E E V D K I V  
 316 CAGAAGTGCGTGGTCAAGAAGGCCATACAGAGGAAACGGATAT  
    Q K C V V K K A I P E E I A Y  
 361 GACACCTTCAAGTGTATTTACGACAGCAAACCTGATTTCTCTCC  
    D I F K C I Y D S K P D F S P  
 406 ATGATTAATTGTTTTGTATTTGACTGAATTTTGACAATAAAGGT  
    I D  
 451 ACTATCGTTATGTAAAAAAAAAAAAAAAAAAAAA

polyadenylation signal

poly (A) tail

Fig. 8.44